Docket No.: 114231.0120 **PATENT**
Customer No.: 21269

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
REID et al.	:	
Serial No.: new application	:	Group Art Unit: unassigned
Filed: Herewith	:	Examiner: unassigned

For: HEPATIC PROGENITORS AND METHODS OF ISOLATING SAME

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please enter this Amendment prior to examination of the application.

IN THE TITLE

Please change the title to the following:

METHODS OF ISOLATING HEPATIC PROGENITORS

IN THE SPECIFICATION

On page 1, please replace the full paragraph under "Cross Reference to Related Applications" with the following:

This Application is a Continuation of Application Serial No. 09/154,222, filed September 16, 1998, allowed; which is a Continuation of Application Serial No. 08/757,336, filed November 27, 1996 and issued as U.S. Patent No. 6,069,005; which is a Continuation of Application Serial No. 08/548,075, filed October 25, 1995, abandoned; which is a Continuation of Application Serial No. 08/155,939 filed November 19, 1993, abandoned; which is a

Continuation-in-Part of Application Serial No. 07/741,128 filed August 7, 1991, entitled PROLIFERATION OF HEPATOCYTE PRECURSORS, abandoned.

On page 12, line 21, please replace the full paragraph with the following:

-- Figure 5 represents a population highly enriched for fetal liver parenchymal cells which was obtained by FACS (R4 cells after exclusion of all OX-43$^-$) and 5 x 10^4 cells/cm^2 plated on type I collagen coated dishes in a serum free, hormonally defined medium. Figure 5A is a phase micrograph showing a typical epithelial colony and very few mesenchymal cells after 4 days in culture (original magnification - 50X). Figure 5B is an indirect in situ immunofluorescence showing incorporation of BrdU in the nuclei of about 25% of the cultured parenchymal cells after 24 hours in culture (original magnification - 50X). Figure 5C is a phase micrograph of panel B; --

IN THE CLAIMS

Please cancel claims 1 to 34 and add the following new claims:

35. (new) A method of enriching for hepatic progenitors from liver comprising:

(a) preparing a suspension of liver cells; and

(b) panning said suspension utilizing antibodies specific for hemopoietic cells, mesenchymal cells, mature liver cells, or combinations thereof, to remove said hemopoietic cells, mesenchymal cells, mature liver cells, or combinations thereof, from said suspension such that said suspension is enriched in hepatic progenitors.

36. (new) The method of claim 35 wherein the mesenchymal cells comprise endothelial cells.

37. (new) The method of claim 35 wherein the mature liver cells comprise at least one of hepatocytes and bile duct cells.

38. (new) The method of claim 35 which further comprises performing multiparametric fluorescence activated cell sorting on said suspension utilizing at least one antibody to a hepatic cell marker, side scatter, forward scatter, autofluorescence, or combinations thereof.

39. (new) The method of claim 35 wherein the antibody specific for hemopoietic cells is a monoclonal antibody.

40. (new) The method of claim 35 wherein said single cell suspension comprises an agent capable of removing calcium from liver cell surface.

41. (new) The method of claim 35 wherein said single cell suspension comprises EGTA.

42. (new) The method of claim 35 wherein said single cell suspension comprises an enzyme capable of dissociating liver cells.

43. (new) The method of claim 35 wherein said single cell suspension contains collagenase.

44. (new) The method of claim 35 wherein said single cell suspension is chilled.

45. (new) The method of claim 35 wherein said single cell suspension is at a temperature of between about 2 and 20 °C.

46. (new) The method of claim 35 wherein the liver is neonatal liver.

47. (new) The method of claim 35 wherein the liver is embryonic liver.

48. (new) The method of claim 35 wherein the liver is adult liver.

49. (new) The method of claim 39 wherein said monoclonal antibody is at least one of OX-43 and OX-44.

50. (new) The method of claim 35 wherein the antibody to a hepatic cell marker is monoclonal antibody 374.3

51. (new) The method of claim 35 wherein said hepatic cell marker is OC.3.

REMARKS

The new claims correct a typographical error in the preamble of an allowed independent claim in application no. 09/154,222, corresponding to claim 35, above, and broaden the scope of the claim. In addition, the title is changed to reflect the claimed subject matter and priority information is provided.

No new matter has been added.

CONCLUSION

Applicants respectfully submit that, upon entry of these amendments and consideration of the Information Disclosure Statement, the instant application is in condition for issue.

Should any questions arise concerning this application, the Examiner is invited to call the undersigned at the number listed below.

AUTHORIZATION

Applicants believe that no fees in addition to the filing fees are due. The Commissioner is hereby authorized to charge any fees which may be required for this Amendment, or credit any overpayment to deposit account #50-0436.

Respectfully submitted,

PEPPER HAMILTON LLP

Gilberto M. Villacorta, Ph.D.
Registration No. 34,038

Thor B. Nielsen, Ph.D.
Registration No. 45,528

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Date: June 5, 2001

Enclosures:
Information Disclosure Statement and PTO-1449
Declaration and Power of Attorney

DC: #186466 v1 3ZVM01!.WPD 114231-115

APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE TITLE:

The title is changed as follows.

[PROLIFERATION OF HEPATOCYTE PRECURSORS] METHODS OF ISOLATING HEPATIC PROGENITORS

IN THE SPECIFICATIONS:

The specification is changed as follows.

On page 1, line 3, please delete the full paragraph under "Cross Reference to Related Applications" and insert in its place, the following:

--This Application is a Continuation of Application Serial No. 09/154,222, filed September 16, 1998, allowed; which is a Continuation of Application Serial No. 08/757,336, filed November 27, 1996 and issued as U.S. Patent No. 6,069,005; which is a Continuation of Application Serial No. 08/548,075, filed October 25, 1995, abandoned; which is a Continuation of Application Serial No. 08/155,939 filed November 19, 1993, abandoned; which is a Continuation-in-Part of Application Serial No. 07/741,128 filed August 7, 1991, entitled PROLIFERATION OF HEPATOCYTE PRECURSORS, abandoned.--

On page 12, line 17, please delete the full paragraph and insert in its place, the following:

-- Figure 5 represents a population highly enriched for fetal liver parenchymal cells which was obtained by FACS (R4 cells after exclusion of all OX-43$^-$) and 5 x 10^4 cells/cm^2 plated on type I collagen coated dishes in a serum free, hormonally defined medium. [Panel A] Figure 5A is a phase micrograph showing a typical epithelial colony and very few mesenchymal cells after 4 days in culture (original

magnification - 50X). [Panel B] Figure 5B is an indirect in situ immunofluorescence showing incorporation of BrdU in the nuclei of about 25% of the cultured parenchymal cells after 24 hours in culture (original magnification - 50X). [Panel C] Figure 5C is a phase micrograph of panel B; --

IN THE CLAIMS:

Claims 1 to 34 are canceled.

Claims 35 to 51 are added as new claims.

--35. (new) A method of enriching for hepatic progenitors from liver comprising:

(a) preparing a suspension of liver cells; and

(b) panning said suspension utilizing antibodies specific for hemopoietic cells, mesenchymal cells, mature liver cells, or combinations thereof, to remove said hemopoietic cells, mesenchymal cells, mature liver cells, or combinations thereof, from said suspension such that said suspension is enriched in hepatic progenitors.

36. (new) The method of claim 35 wherein the mesenchymal cells comprise endothelial cells.

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38. (new) The method of claim 35 which further comprises performing multiparametric fluorescence activated cell sorting on said suspension utilizing at least one antibody to a hepatic cell marker, side scatter, forward scatter, autofluorescence, or combinations thereof.

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50. (new) The method of claim 35 wherein the antibody to a hepatic cell marker is monoclonal antibody 374.3

51. (new) The method of claim 35 wherein said hepatic cell marker is OC.3. --

Docket No.: 114231.120 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application :
:
Inventor: REID et al. :
: Group Art Unit: Unassigned
Serial No. new application :
: Examiner: Unassigned
Filing Date: Herewith :

For: HEPATIC PROGENITORS AND METHODS OF ISOLATING SAME

SUBMISSION OF SUBSTITUTE DRAWINGS

Honorable Commissioner of
Patents and Trademarks
Washington, D. C. 20231

Sir:

Applicants submit herewith substitute drawings for Figs.1-11 (11 Sheets) that were submitted with prior application Serial No. 09/154,222 in compliance with 37 CFR § 1.121 (3)(i)-(ii).

No fee is required for this submission. If any fee is required, the commissioner is authorized to charge the fee to Deposit Account No. 50-0436.

Respectfully submitted,
PEPPER HAMILTON LLP

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Thor B. Nielsen
Thor B. Nielsen, Ph.D.
Registration No. 45,528

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Date: June 5, 2001
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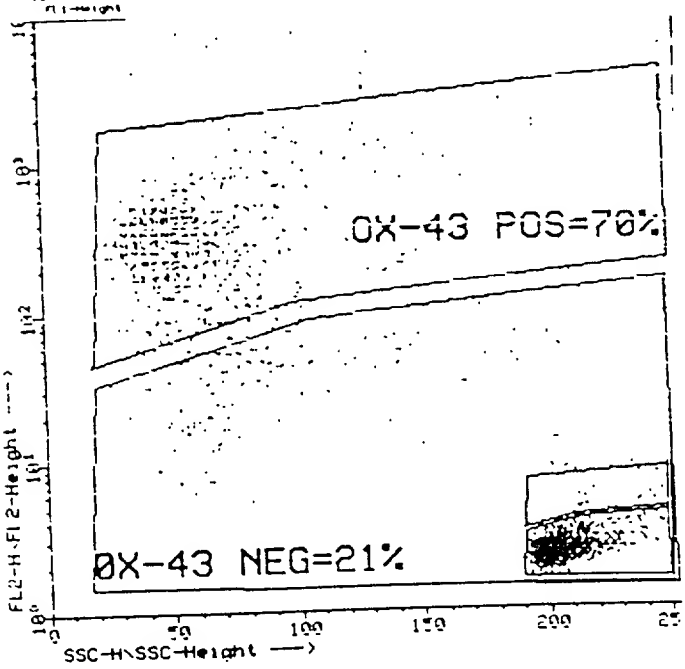
Figure 1

A

B



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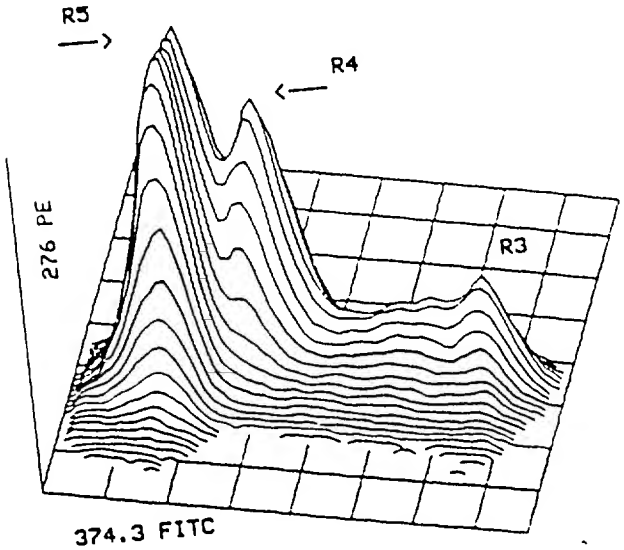
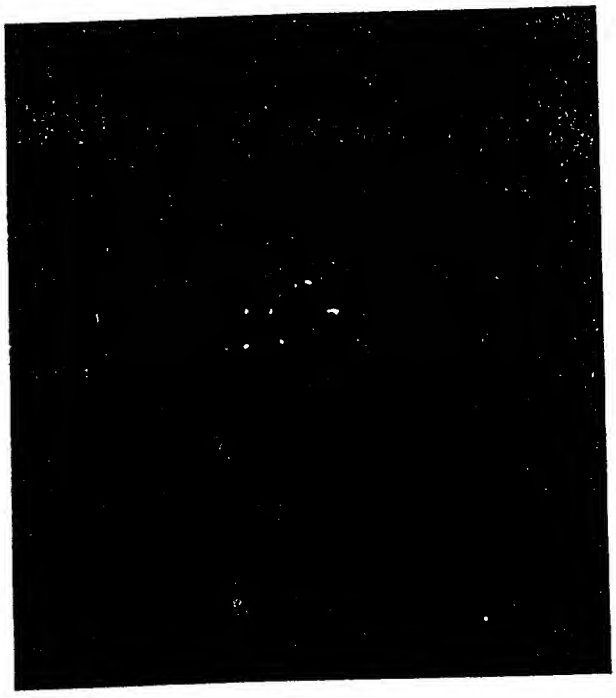
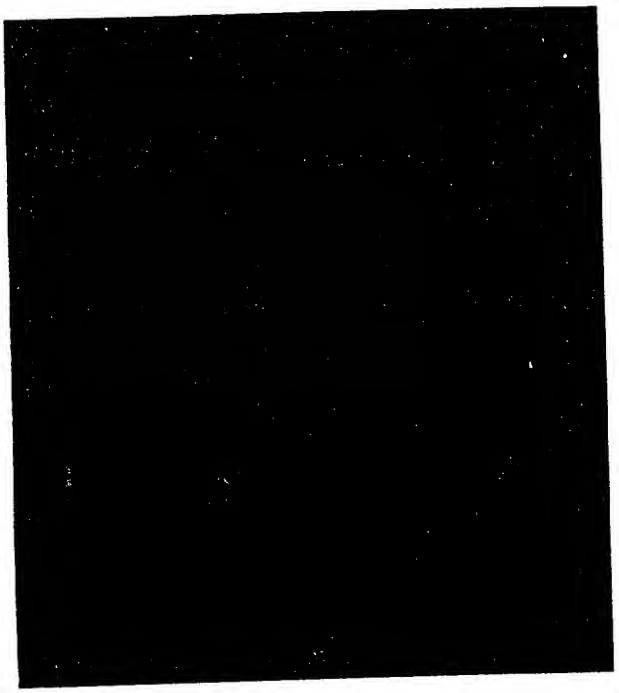


Figure 2

A



B



C

Figure 3

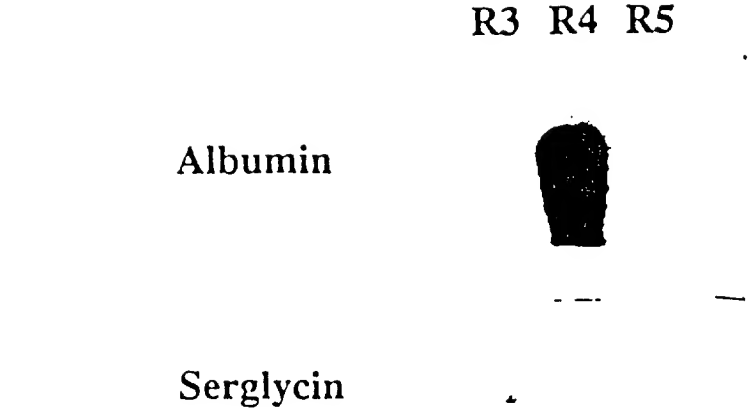


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Figure 4

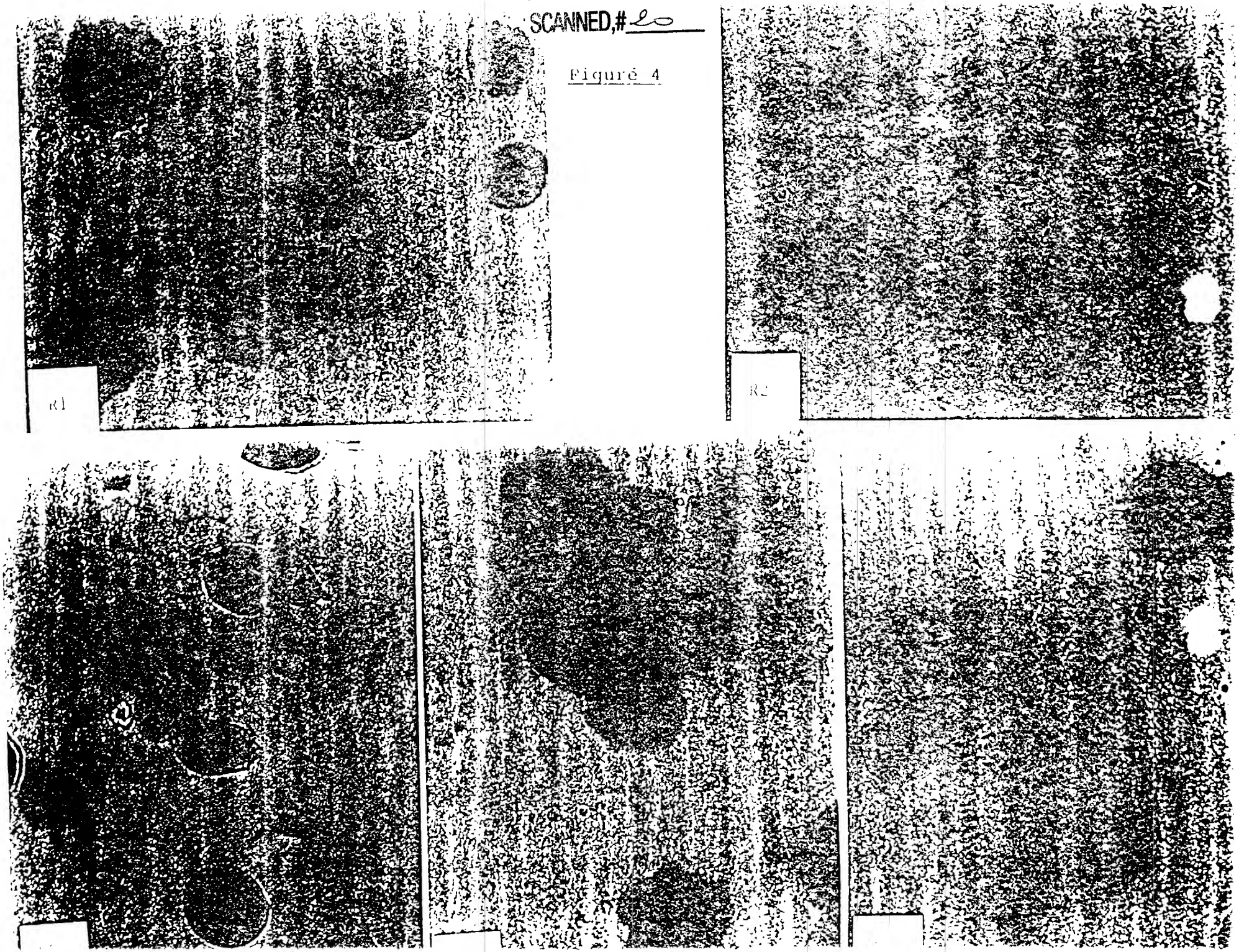

Figure 5

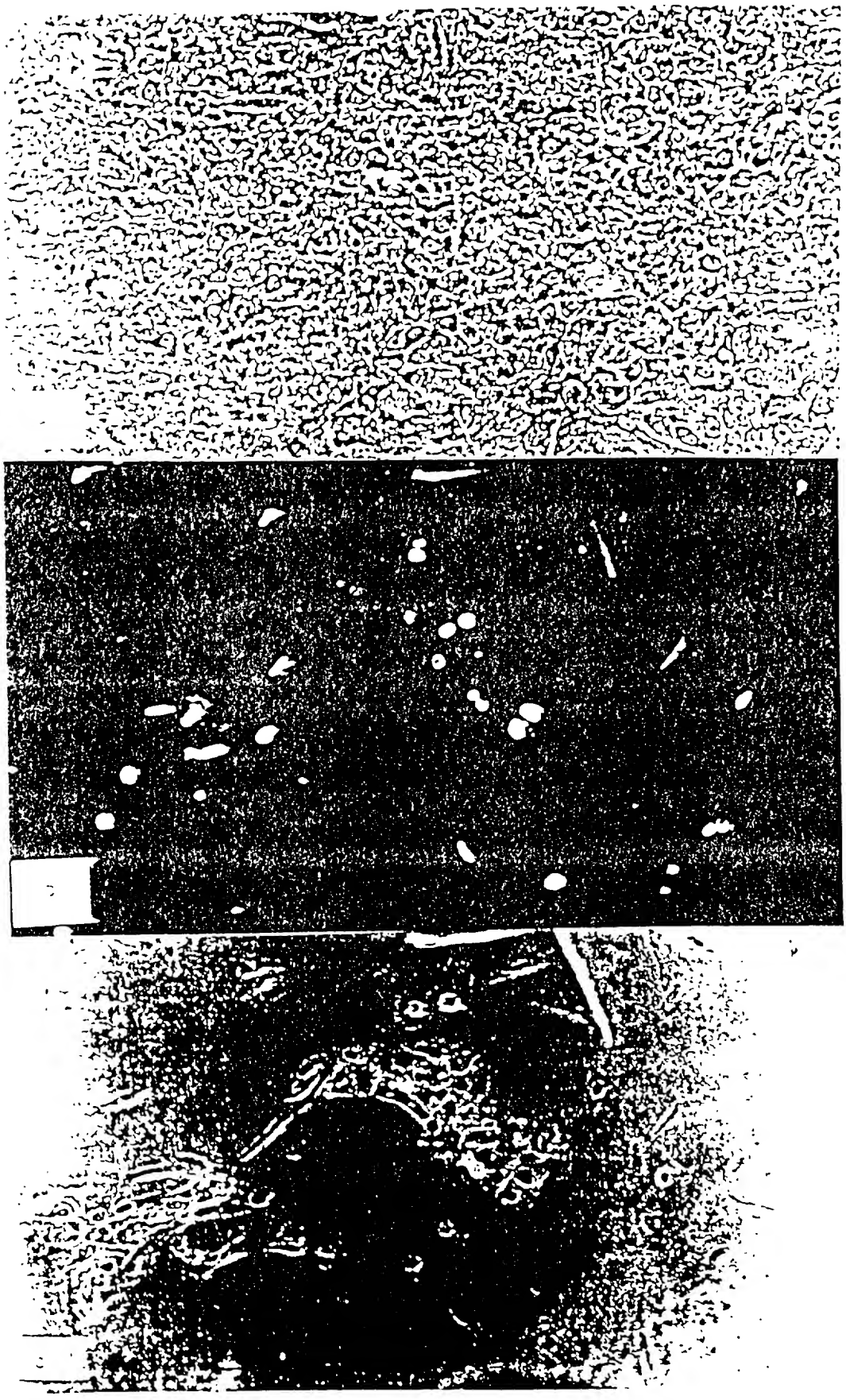

Figure 6

Flow Diagram of Hepatoblast Enrichment

Livers (8-9 mgs)

↓ Dispersion with EGTA and then collagenase

Single Cell Suspension Preparation: Collagenase, EGTA, 4°C

↓ 10^7 cells/8 mgs liver

↓ 3.2 ± 1.3% are ALB^+

↓ 2.5 ± 0.7% are AFP^+

↓ 87.9 ± 2.5% are $OX43/44^+$

Panning

Red Blood Cell Panning (2X)

↓ 29 ± 5% of cells remain

↓ 9.5 ± 1.2% are ALB^+

↓ 9.8 ± 0.9% are AFP^+

↓ 80.4 ± 3.9% are $OX43/OX44^+$

OX-43/OX-44 Panning (myeloid and endothelial cells)

↓ 16 ± 4% of cells remain

↓ 14.8 ± 3.6% are ALB^+

↓ 14.9 ± 2.5% are AFP^+

↓ 69 ± 10% are $OX43/OX44^-$

Fluorescence Activated Cell Sorting

Negatively Sort for Contaminant Cell Populations:

OX-43 (CD)/OX-44 (CD37)$^+$ Cells = precursors and mature forms of hemopoietic cells (myeloid, erythroid) and endothelial cells

Of remaining cells (OX-43$^-$ + OX-44$^-$ cells), sort for cells varying in OC.3 expression and granularity:

OX-43/(CD)/OX-44 (CD37)$^-$ Cells = mostly hepatic precursors, some residual hemopoietic cell contaminants, stromal cells

OC.3$^+$, granular cells = committed bile duct precursors (AFP^+, ALB^-)

OC.3$^-$, granular cells = committed hepatocyte precursors (AFP^+, ALB^{+++})

OC.3$^+$ agranular cells = early hepatoblasts (AFP^{+++}, albumin$^+$ and CK 19$^-$)

Figure 7

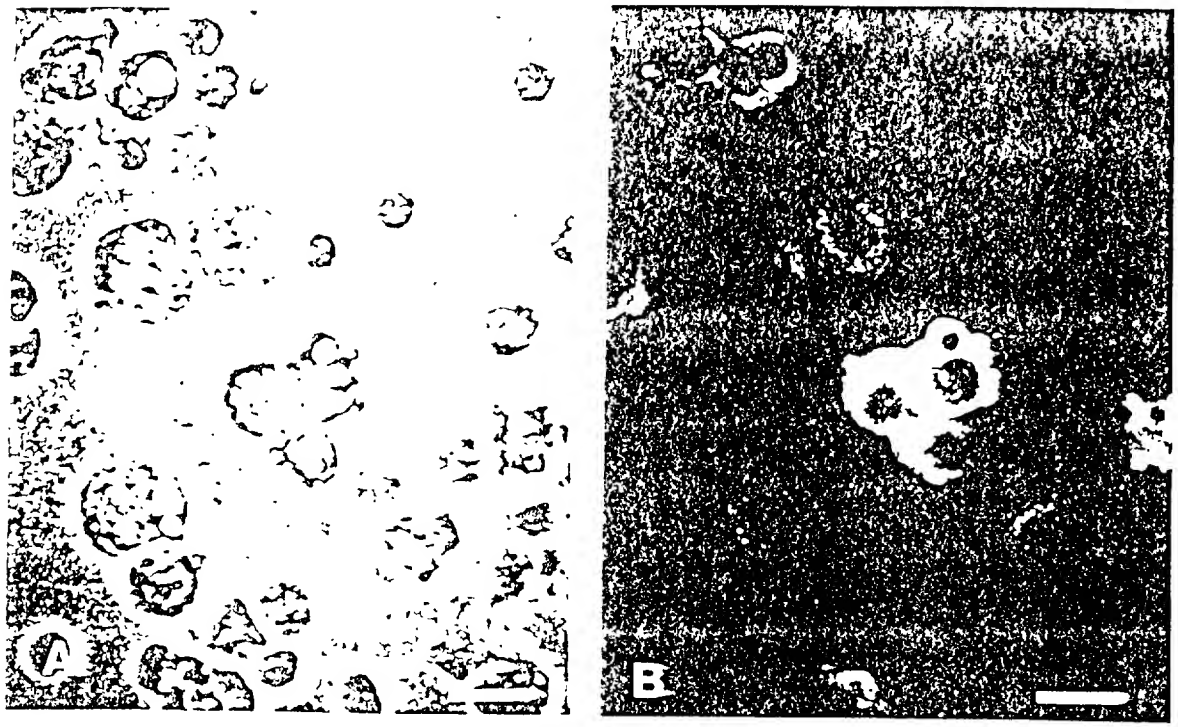

Figure 8

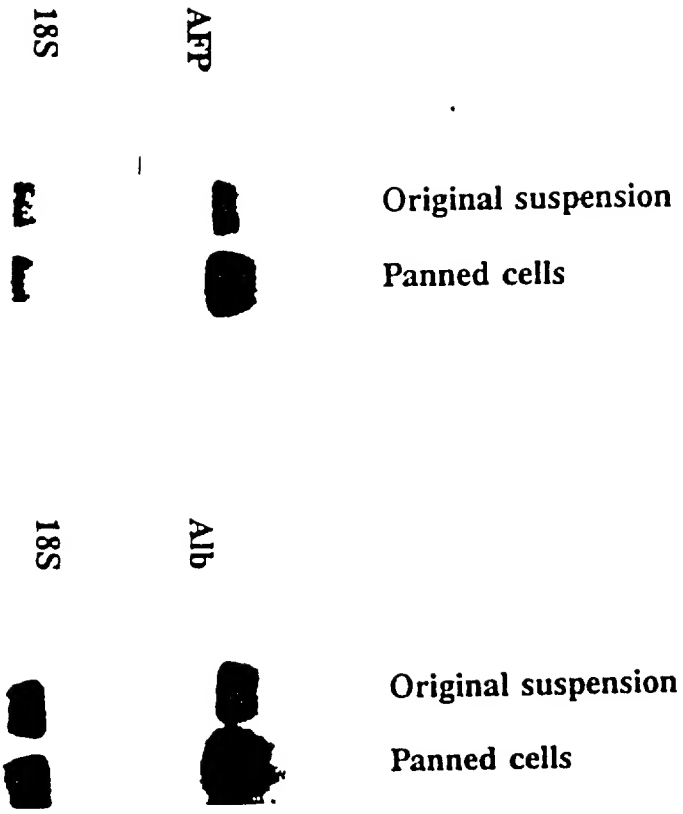

Figure 9

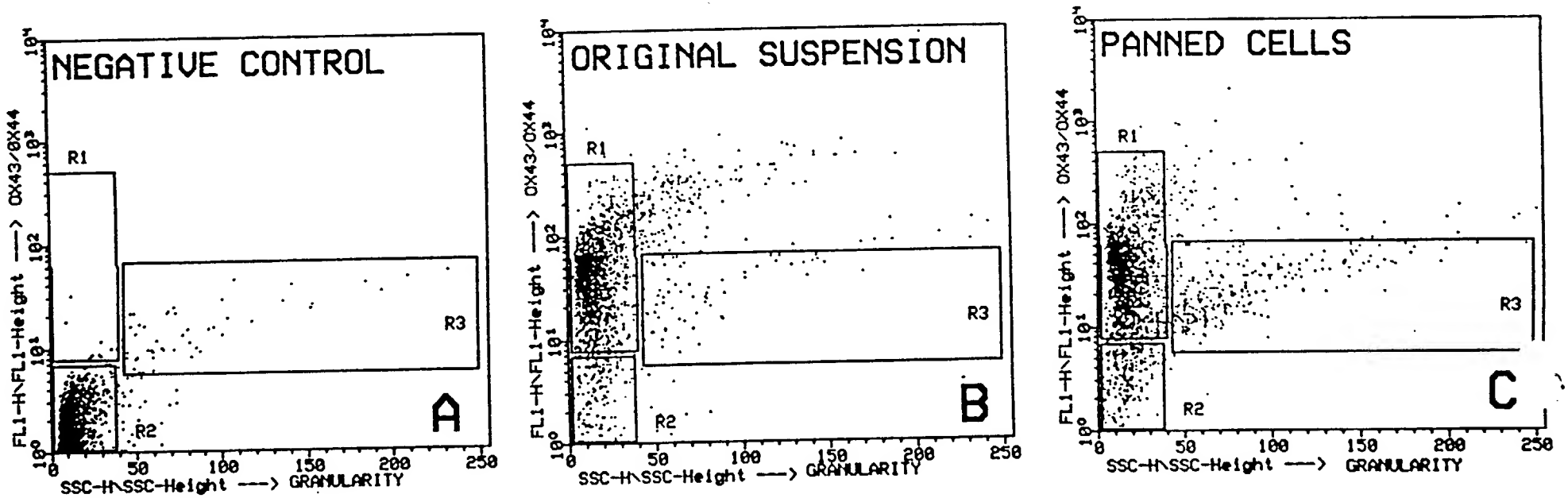

Figure 10

Figure 11